

THE EFFECT OF STOCKING DENSITY ON GROWTH AND SURVIVAL OF THE FRESHWATER MUSSEL *UNIO TIGRIDIS* (BIVALVIA: UNIONOIDEA)

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ABSTRACT

The effects of four stocking densities of 20, 40, 60, 80 mussels/m² on growth of *Unio tigridis* were investigated. The growth of the freshwater mussel *U. tigridis* in cages was studied for a year from March 2003 to April 2004 in Lake Gölbaşı. Juvenile mussels with the initial weight of 0.64 ± 0.08 g were stocked in metal cages placed on the lake bottom. Growth in live weight, shell length, width and height was measured monthly for the four different stock groups. Chlorophyll a, magnesium (Mg), nitrite (NO₂), nitrate (NO₃), amount of organic matters, ammonia (NH₃), phosphate (PO₄), silica (Si) and calcium (Ca) were also analyzed monthly. All water quality parameters were within proper limits for mussel culture. At the end of the study, the greatest live weight (5.55 ± 0.03 g) was obtained from 40 mussels/m², with 3.52 ± 0.02 cm and 1.47 ± 0.03 cm as length and width values, respectively. The greatest height was determined in a stocking rate of 60 mussels/m², with 1.99 ± 0.03 cm. Therefore, juveniles should be stocked in early spring for *U. tigridis* at a level of 40–60 individuals/m².

Key words: *Unio tigridis*, freshwater bivalve, mussel culture, stocking density, growth.

INTRODUCTION

Bivalve molluscs are dominant filter-feeders on most of the planktonic biomass and exert control over ecosystem structure and function (Irmgard et al., 2000). Filtration by bivalves can lead to a large decreases in phytoplankton and other particles in the water column (Welker & Walz, 1998). Freitas et al. (2003) reported that growth limits in natural populations of bivalves is related to diminished food availability. Freshwater burrowing bivalves of the order Unionoida (families Unionidae, Margaritiferidae, Hyriidae, Iridinidae and Mycetopodidae) and Veneroida (families Corbiculidae and Sphaeriidae) have the potential to greatly influence freshwater ecosystem processes (Vaughn & Hakenkamp, 2001).

Freshwater mussels of the family Unionidae need to be protected because many species are threatened by extinction (Strayer et al., 2004). The global decline of freshwater mussel populations in the last five decades has caused much concern among national and international conservation organizations (Araujo & Ramos, 2000). In contrast with other unionid species, *U. tigridis* is not threatened by extinction due to environmental hazards and/or commercial use.

Unio tigridis (BOURGUIGNAT, 1852) (Unionidae) is ecologically important in southeastern Turkey (Schütt, 1983; Şereflişan, 2003, 2005, 2008). Information on the ecological importance of this species is very limited, and there have been no ecological studies of this species in Turkey. We have a few personnel communications about the ecology of *U. tigridis* in Iraq. *Unio tigridis* is benthic and lives on substrata at a depth of 1–5 m. Individuals of this species have been observed in both lakes and irrigation channels. The life cycle of unionids is remarkable and complex, with microscopic larvae of unionids (glochidia) having hooks to attach themselves to the fins or gills of host fish (Şereflişan et al., 2009). Therefore, freshwater mussels' survival depends not only on abiotic habitat conservation but also on the availability of the host fish (Araujo & Ramos, 2000; Barnhart, 2006).

Unio tigridis can grow to 8 cm long and may be considered for aquacultural purposes. Also, in souvenir markets, the nacreous layers of its shells have been used for inlays in furniture (Şereflişan, 2008). However, there have been no studies on the environmental requirements, growth and survival rates in cages in this species. Moreover, there has been no effort to culture of

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this species. Therefore, the purpose of this study was to assess the potential to culture *U. tigridis* as an alternative human food source.

Culture studies carried out on other unionids have shown differences in growth rates at different locations (Mueller & Patzner, 1996; Paterson & Nell, 1997). These differences were associated with environmental factors such as chlorophyll a, organic matter, pH and temperature (Morris & Corkum, 1999). It is now agreed that the type of aquaculture system, the stocking density, and depth should be considered among many important factors defining an aquacultural strategy for juvenile and adult stages (Taylor et al., 1998). The effects of these factors are clearly reflected by growth rate and survival (Monteforte & Morales, 2000). Additionally, temperature affects the duration of the growing season (Morris & Corkum, 1999).

To date, a great deal of research has been carried out on the biology, ecology and distribution of unionids (Strayer et al. 1994; Çek & Şereflişan, 2006). There are few publications on the culture of unionids (Lima et al., 2006), and there has been no effort to culture of *U. tigridis*. The present study was designed to determine the effect of four stocking densities on both growth and survival of this species over a 12-month period. Preliminary information about the culture of this species will be of importance for freshwater mussel aquaculturists throughout the world.

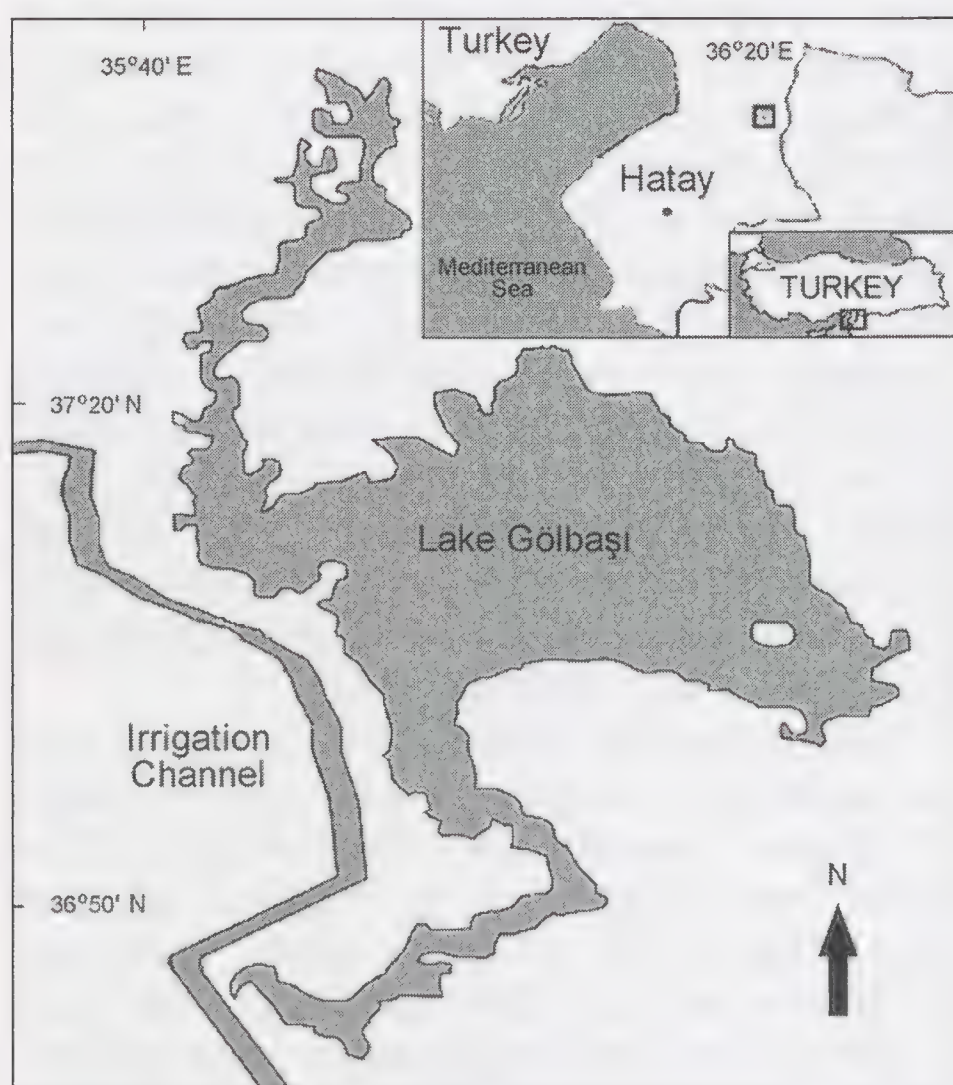


FIG. 1. Map of the study site.

MATERIAL AND METHODS

Study Site

The experiment was carried out in Lake Gölbaşı, Hatay, southern Turkey, between May 2001 and April 2002 (36°30'16"N, 36°29'42"E). Lake Gölbaşı is in the eastern Mediterranean region of Turkey, 50 km north of the city of Antakya (36°29'E, 36°30'N). The lake is a natural lake with a surface area of 12 km² at altitude of 80 m. The lake volume is 8,000,000 m³, with a maximum depth of 6 m. Since it supplies water to the surrounding cotton fields, the water level is lower in spring and summer. The lake has no incoming or outgoing creeks and is only fed by spring water (Fig. 1).

Environmental Factors

Oxygen, temperature and pH were measured directly in the field with digital instruments. Oxygen and temperature were measured by YSI model 52 oxygen meter and pH by Orion model 420A pH meter. Nansen-type bottles were used for sampling for lake water. One l water samples were taken to laboratories of Mustafa Kemal University to analyze chlorophyll a (Ch a), particulate organic matter (POM), ammonia (NH₃), nitrite (NO₂), nitrate (NO₃), calcium (Ca), magnesium (Mg), phosphate (PO₄) and silica (Si). Ch a and POM samples were passed through a 150 µm nylon mesh to remove large particles. The number of particles was determined with a Fluorometrik method (APHA, 1980). The EDTA method was used to analyze calcium (Ca), magnesium (Mg), whereas a spectrophotometer was employed to analyze, ammonia (NH₃), nitrite (NO₂), nitrate (NO₃), phosphate (PO₄) and silica (Si) (APHA, 1971).

Biological Material

Juveniles were collected with a scoop net and a hand dredge at a depth of 1–3 m in Lake Gölbaşı. The initial main shell measurements were 6.2 ± 0.08 mm in length, 4.0 ± 0.01 mm in height and 2.5 ± 0.02 mm in width. The average initial weight of the juvenile mussels was 0.64 ± 0.08 g. Juveniles were stocked in cylindrical cages of one cubic m volume (115 cm diameter and 25 cm height). Cages were covered with a metal net (2 cm mesh size), and a polyamide net with 6 mm mesh size was placed on the inside of the metal net. Experimental design was four treatments with three replicates each.

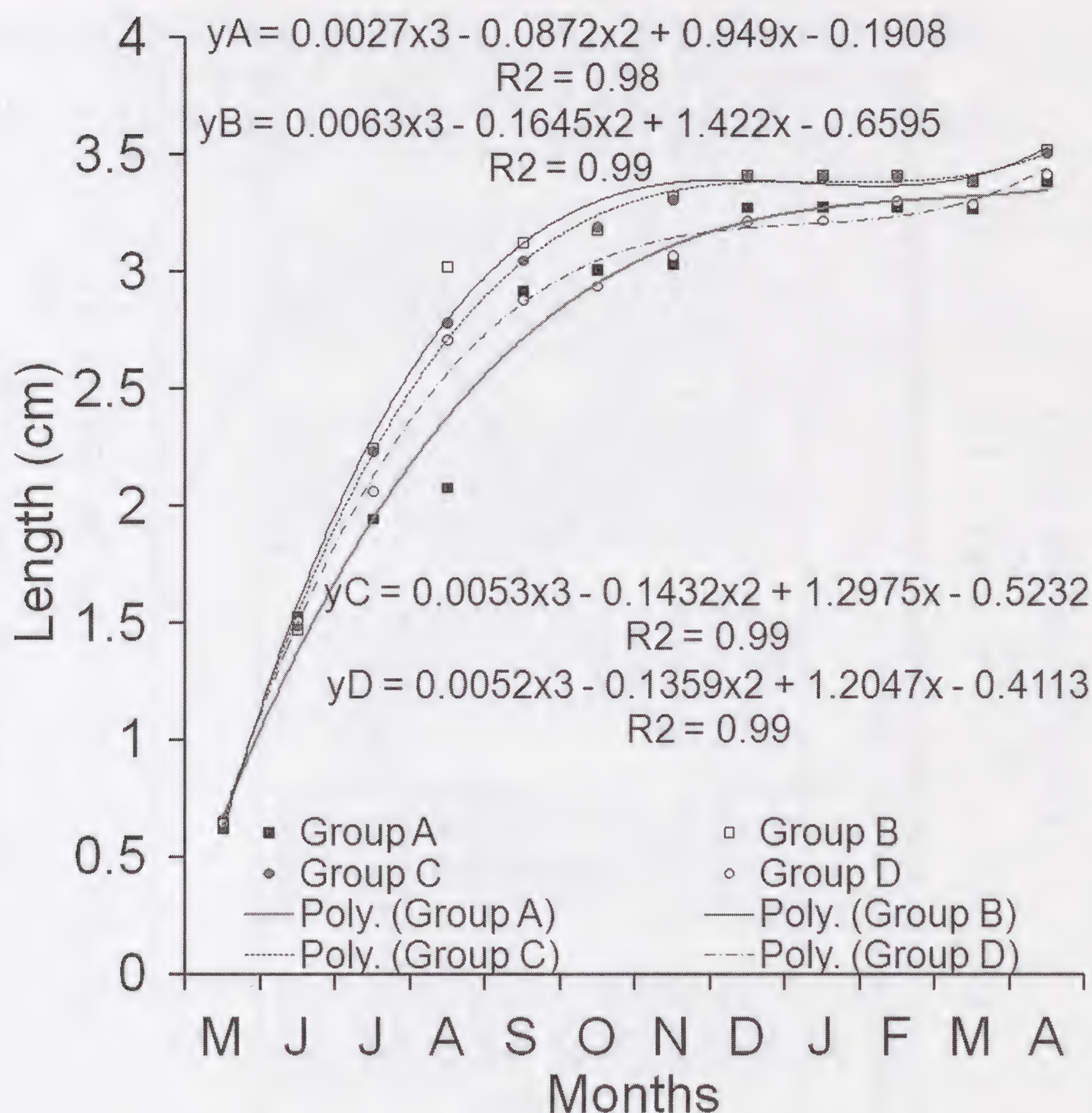


FIG. 2. Mean growth in shell length of *Unio tigridis* grown at various densities for 12 months.

Four stocking densities were used: group A with 20 mussels/m², group B with 40 mussels/m², group C with 60 mussels/m², and group D with 80 mussels/m². The juvenile mussels were placed in approximately 2 m depth in 12 metal cages.

Sampling and Biometry

Systematic sampling was conducted, and all mussel measurements were made according to Hoggarth (1999). Shell length, width, height and live weight measurements were taken monthly for 12 months, while wet meat weight measurements were determined at the end of the study. Measurements were performed

by sampling of 20 mussels from each cage randomly. Mantle cavity water of the mussels sampled was drained before weighing. The growth parameters were estimated from the changes in shell length (L), live weight (LW) and wet meat weight (WMW). Mortality was determined by counting the number of empty shells in each cage monthly.

Statistical Analyses

Analysis of variance (ANOVA) was employed to detect growth differences between the stocking groups. Significance level was selected as 0.05 to measure statistical differences between treatment groups.

TABLE 1. Water quality parameters measured for a duration of 12 months in the Lake Gölbaşı of Turkey (mg l⁻¹) (mean ± SE); POM: particulate organic matter, NO₂: nitrite, NH₃: ammonia, NO₃: nitrate, PO₄: phosphate, Mg: magnesium, Si: silica, Ca: calcium.

Months	Chlorophyll a	POM	NO ₂	NH ₃	NO ₃	PO ₄	Mg	Si	Ca
May	0.83±0.01 ^{ef}	1.98±0.02 ^{de}	0.008±0.00 ^a	0.23±0.01 ^c	1.36±0.00 ^c	0.02±0.00 ^a	2.79±0.03 ^{ab}	4.67±0.01 ^c	4.48±0.02 ^a
June	0.78±0.01 ^e	2.03±0.04 ^{def}	0.009±0.00 ^a	0.39±0.01 ^e	1.40±0.01 ^d	0.02±0.00 ^a	2.76±0.05 ^a	3.06±0.00 ^{ab}	4.55±0.04 ^{ab}
July	0.36±0.02 ^b	2.45±0.01 ^g	0.017±0.00 ^c	0.28±0.01 ^{cd}	1.35±0.01 ^c	0.03±0.00 ^b	2.84±0.05 ^{bcd}	3.21±0.01 ^{ab}	4.55±0.04 ^{ab}
August	0.32±0.01 ^b	1.39±0.03 ^b	0.013±0.00 ^b	0.11±0.01 ^b	1.32±0.00 ^b	0.05±0.01 ^b	2.81±0.02 ^{bc}	9.47±0.01 ^e	4.45±0.03 ^a
September	0.18±0.01 ^a	2.46±0.01 ^g	0.018±0.00 ^c	0.07±0.00 ^a	1.31±0.01 ^{ab}	0.01±0.00 ^a	2.95±0.05 ^{ef}	2.98±0.01 ^a	4.56±0.03 ^{ab}
October	0.47±0.02 ^d	1.09±0.02 ^a	0.014±0.00 ^b	0.07±0.00 ^a	1.30±0.00 ^a	0.01±0.00 ^a	2.74±0.04 ^a	5.68±0.00 ^{cd}	4.46±0.02 ^a
November	0.42±0.03 ^{bc}	1.25±0.03 ^b	0.013±0.00 ^b	0.09±0.01 ^a	1.29±0.00 ^a	0.01±0.00 ^a	2.92±0.02 ^{de}	5.69±0.01 ^{cd}	4.56±0.05 ^{ab}
December	0.43±0.01 ^{bc}	1.53±0.02 ^c	0.009±0.00 ^a	0.08±0.01 ^a	1.29±0.00 ^a	0.01±0.00 ^a	2.93±0.02 ^{de}	5.48±0.00 ^{cd}	4.55±0.03 ^{ab}
January	0.48±0.02 ^{cd}	1.68±0.03 ^{cd}	0.007±0.00 ^a	0.07±0.01 ^a	1.28±0.01 ^a	0.01±0.00 ^a	2.95±0.03 ^{ef}	5.23±0.01 ^{cd}	4.53±0.02 ^{ab}
February	0.53±0.02 ^{cd}	1.78±0.01 ^{cde}	0.004±0.00 ^a	0.06±0.01 ^a	1.28±0.01 ^a	0.01±0.00 ^a	2.98±0.02 ^{ef}	5.11±0.01 ^{cd}	4.45±0.01 ^a
March	0.79±0.01 ^e	1.83±0.02 ^{cde}	0.003±0.00 ^a	0.08±0.01 ^a	1.29±0.01 ^a	0.01±0.01 ^a	2.89±0.04 ^d	5.02±0.00 ^{cd}	4.41±0.02 ^a
April	0.82±0.02 ^{ef}	1.92±0.01 ^{cde}	0.006±0.00 ^a	0.12±0.01 ^b	1.32±0.00 ^b	0.02±0.01 ^a	2.83±0.02 ^{bcd}	4.96±0.00 ^c	4.42±0.01 ^a

a, b, c, d, e, f Letters show statistical differences in vertically (*P* < 0.05).

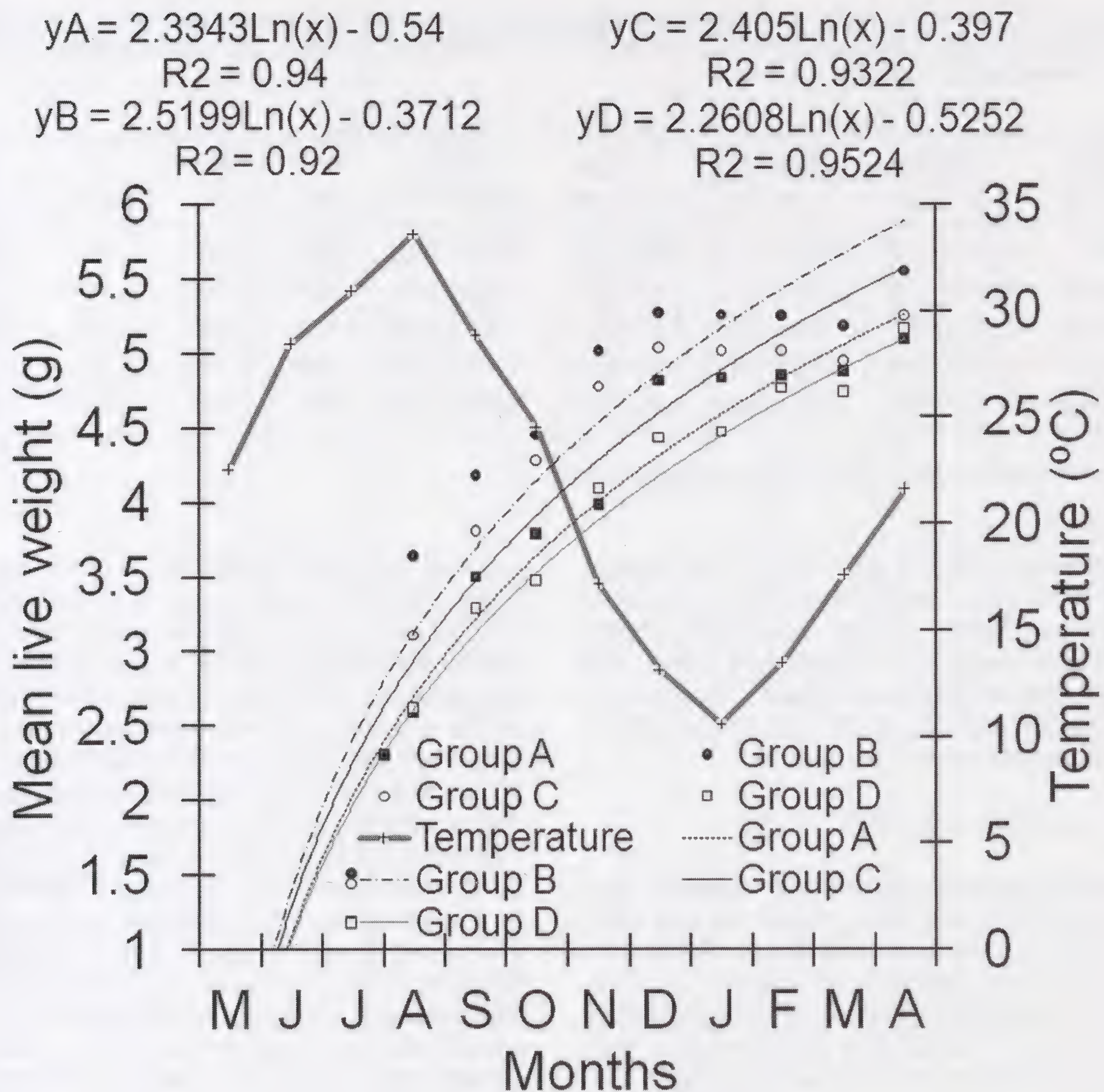


FIG. 3. Growth and temperature relationship of *Unio tigridis* grown at different densities for 12 months.

Also, growth-time regressions were calculated by estimation curves (Fig. 2). These curves are useful in estimating the appropriate data model. For this purpose, linear, logarithmic, inverse, quadratic, cubic, power, compound and exponential curves were tested to determine the best regression model. The results of variance analysis, the lowest standard error and regression coefficients were also evaluated to find best regression model. Zar (1996) was used to compare the regression equations of the models. The Excel program was used to determine the mean and standard errors for water quality parameters. All statistical analyses were carried out with the SPSS program.

RESULTS

Environmental Factors

The temperature ranged from 10.59°C in January to 33.63°C in August. Annual range of oxygen concentration was 4.51 ppm (January) to 9.60 ppm (November). The changes in pH were nearly insignificant during the course of the study. Monthly distributions of chlorophyll a (Ch a), particulate organic matter (POM), ammonia (NH₃), nitrite (NO₂), nitrate (NO₃), calcium (Ca), magnesium (Mg), phosphate (PO₄) and silica (Si) are shown in Table 1. Monthly distributions of Ch a, Ca, and Mg were

TABLE 2. The mean ($\bar{x} \pm SD$) characteristics of freshwater mussel (*Unio tigridis*) cultivated in cage and shell ratios [Group A (20 mussels/m²), Group B (40 mussels /m²), Group C (60 mussels/m²), Group D (80 mussels/m²)].

Stock Groups	Weight (W in g)	Length (L in cm)	Height (H in cm)	Width (W in cm)	W (g)/L	W (cm)/L	W (cm)/H	H/L
Initial	0.64 ± 0.08	0.62 ± 0.08	0.40 ± 0.01	0.25 ± 0.02	1.03 ^a	0.40 ^{ab}	0.62 ^a	0.64 ^a
Group A	5.10 ± 0.05	3.39 ± 0.02	1.88 ± 0.01	1.32 ± 0.02	1.50 ^b	0.39 ^a	0.70 ^{ab}	0.55 ^b
Group B	5.55 ± 0.03	3.52 ± 0.02	1.96 ± 0.02	1.47 ± 0.03	1.57 ^c	0.42 ^b	0.75 ^b	0.55 ^b
Group C	5.24 ± 0.05	3.50 ± 0.03	1.99 ± 0.03	1.47 ± 0.02	1.49 ^b	0.42 ^b	0.73 ^b	0.56 ^b
Group D	5.16 ± 0.04	3.42 ± 0.03	1.89 ± 0.01	1.35 ± 0.04	1.50 ^b	0.39 ^a	0.71 ^{ab}	0.55 ^b

a, b, cLetters show statistical differences in vertically ($P < 0.05$).

significantly different ($P < 0.05$). The highest Ch a (0.83 ± 0.01 mg/L) and NO₃ (1.40 ± 0.01) levels were in May, and the highest NH₃ (0.39 ± 0.01) level was in June. POM (2.46 ± 0.01) and NO₂ (0.018 ± 0.00) were highest in September, and the highest PO₄ (0.05 ± 0.01) and S (9.47 ± 0.01) levels were in August.

Shell and Tissue Growth

Mean characteristics of these mussels, such as weight (W), length (L), height (H) and width (W) are summarized in Table 2. All groups showed fast growth from May to October. Length increment differed significantly among the groups ($P < 0.05$). The growth in length was determined to be the highest in group C (60 mussels/m²) (Fig. 2). It was determined that both stocking density and water temperature affected mussel growth (Fig. 3). Wet meat weight was highest in the stocking group B of 40 mussels/m², and wet meat yields of 20 mussels/m² and 40 mussels/m² were found to be higher than that of the stocking groups of 60 and 80 mussels/m² (Fig. 4a). A negative growth rate was observed in winter months, followed by a quick recovery in spring. Positive significant relationships were found between width-length and width-height ratios for all groups ($P < 0.05$).

DISCUSSION

Older reports, such as Coker et al. (1921), and more recent research on unionids (Beaty & Neves, 1996; Hoggarth 1999; Morris & Corkum, 1999) are essential to have an understanding of the main growth characteristics of freshwater

mussels. In culture conditions, stocking density is an important factor causing growth differences in live weight, wet meat weight and shell growth (Sereflisan, 2003). It was determined that stocking densities had a significant effect on the shell length, wet meat weight and live weight growth of *U. tigridis* for the duration of 12 months ($P < 0.05$). Considering all growth characteristics, the best growth was achieved in group B (40 mussels/m²). Growth characteristics of mussels were expected to be higher for the lower stocking densities compared to the higher densities due to less competition (Mackie, 1984). Our results, however, indicated that there was not an inverse relation between growth and stocking density. This mode of growth pattern may be related to such behavioral characteristics as proper site selection and food search, and protection against predators. Also, Mackie (1984) reported colonial living and higher growth for the freshwater mussels at a particular stocking density and supported our findings. There has been no study focused on the effect of stocking density on survival of *U. tigridis*. In our study, it was determined that stocking densities had no effect on survival rate. In an earlier study, survival rates of the Asian freshwater mussel *Limnoperna fortunei* stocked at the different rates were not significant and showed high survivorship for all groups (95%) at the end of the trial (Boltovskoy & Cataldo, 1999).

The greatest growth of shell length was determined to be in spring and summer months in group C (60 mussels/m²). However, it was determined that shell length growth of *U. tigridis* was better in warmer months than that in the cold months for all stocking groups. The Asian freshwater mussel *Limnoperna fortunei*

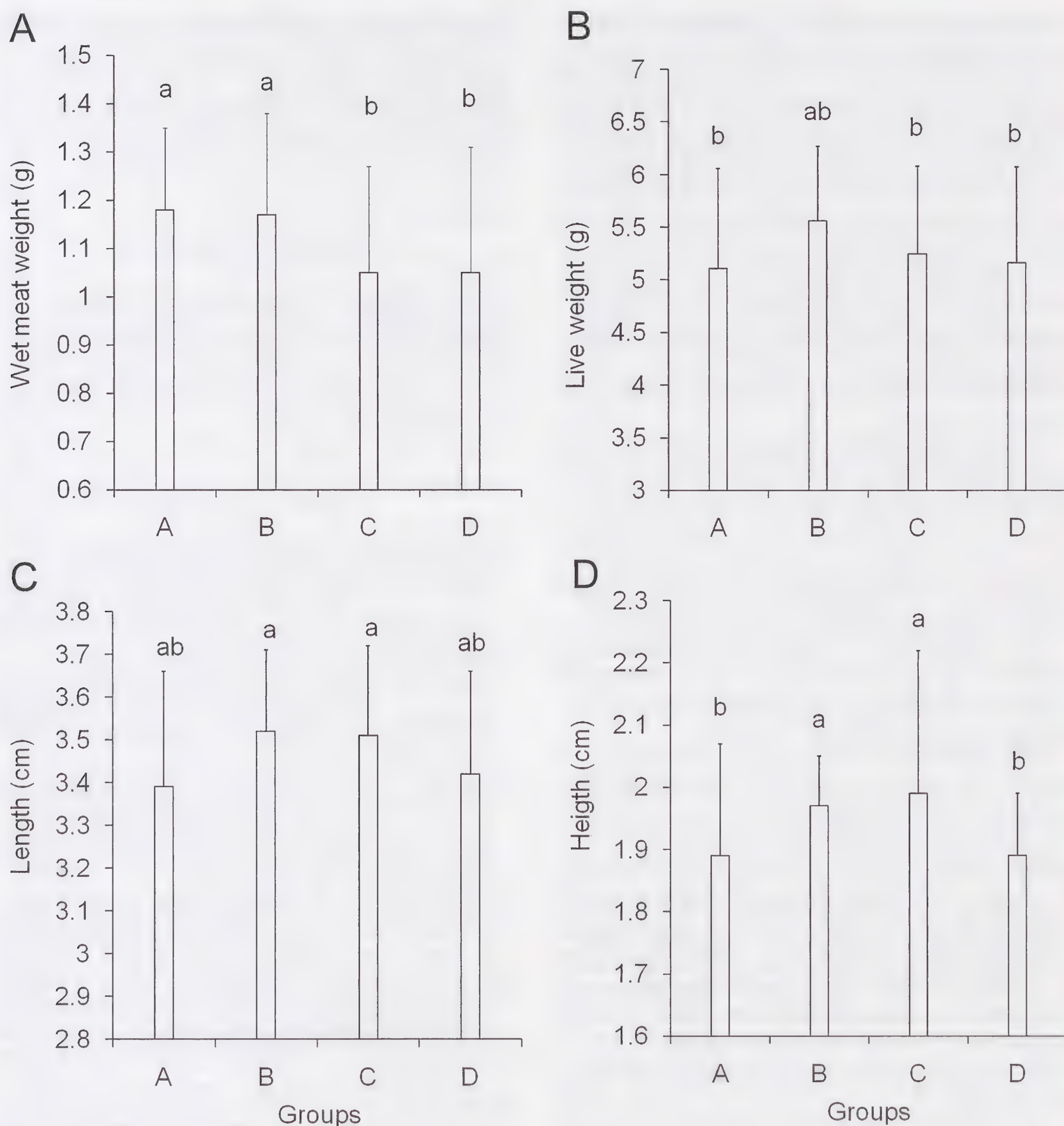


FIG. 4. Growth of each group of *Unio tigridis* as wet meat weight (A), live weight (B), length (C) and height (D) grown at different densities for 12 months.

also grew better in warm than in cold months (Boltovskoy & Cataldo, 1999) at different stocking densities. Also, Mallet et al. (1987) indicated that the rate of shell length and wet meat weight increases in mussels are greater in warm regions than in cold regions.

Oxygen and pH values are as important as temperature and have a profound effect on all living organisms (Morris & Corkum, 1999; Karayücel & Karayücel, 2000b). In our study, pH and oxygen values were changed by tem-

perature. Unionids are able to live at a pH of 4.7 (the minimum level) and to reproduce and grow at pH values between 5.6–8.3 (McMahon, 1991). pH levels of our study are within the range of $7.78 \pm 0.04 - 8.02 \pm 0.01$, which is in accordance with the literature. The fertility of natural waters generally increases with the increase in total hardness (Tepe & Boyd, 2003; Tepe, 2009). Hard waters often have moderate to high total alkalinities, are well buffered at a neutral to slightly alkaline pH, and tend to be

more productive than poorly mineralized, acidic waters (Boyd & Tucker, 1998). Nitrate-nitrogen ($\text{NO}_3\text{-N}$) level (1.01–1.43 mg/L) was within the optimum range for living biomass in our study. The low nitrite-nitrogen ($\text{NO}_2\text{-N}$) value in our study indicated that the lake water has enough oxygen for nitrification. Organic matter concentration in lake water varied between 1.23–3.22 mg/L. This variation might be due to precipitation and runoff. The highest silica value in the lake was 9.47 ± 0.01 in August. The silica level can be low during spring when algae population is the highest, and this level may also show differences depending on season, water flow and precipitation.

Despite the fact that mussel growth is strongly related to annual primary productivity, it is not as related with chlorophyll a concentration (Small & Van Stralen, 1990). In shallow lakes, increased phytoplankton and zooplankton levels do not mean that the season has a direct effect on the chlorophyll a level (Adrian et al., 1999). Our findings, confirming to the literature, showed that the mussel growth levels were the highest in August, and the chlorophyll a levels were the highest in April and May.

Transparency, viscosity and hardness of lakes and rivers can change due to springs or other incoming waters, but there was no incoming water source in our study area. Low growth even atrophy can be seen in mussels in waters with a low calcium concentrations (McMahon, 1991). Calcium is vital for mussel shell deposition (McMahon, 1991). Mallet et al. (1987) reported that the accumulation of calcium in mussel shells during the juvenile stage is very rapid, and shell growth in contrast to meat growth does not depend on food availability.

Other factors influencing mussel growth are water depth and bottom structure, which can impact the growth of the unionid *Anodonta cygnea* (Mueller & Patzner, 1996). The growth decreases in deeper water, and mussels placed in the baskets cultivated in sediment grew better than those on rocky or other hard substrata (Paterson & Nell, 1997). The present study was performed on the sediment at a depth of 1.5–2.0 m, and these conditions were optimum for the culture of this species.

The present study gives preliminary information on the effect of stocking density on growth of *U. tigridis* cultured in cages for 12 months. Also, we examined the some water quality criteria influencing primary productivity of the lake. Since highest live weight and shell height

were obtained from the stocking groups of 40 mussels/m² and 60 mussels/m² in spring and summer, it is advisable to stock juveniles in early spring for *U. tigridis* at a level of 40–60 individuals/m². Other unionids should be similarly evaluated in future investigations.

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